



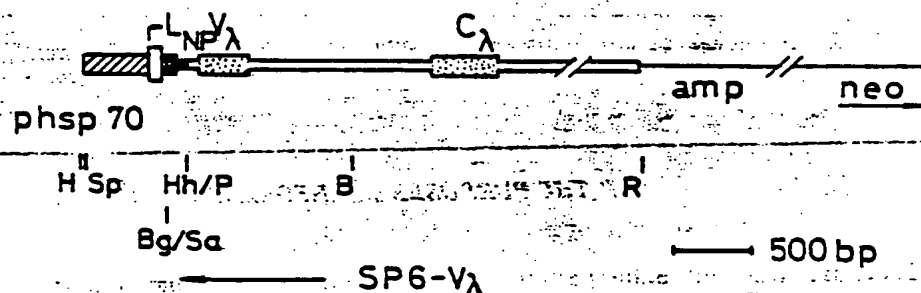
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UNDER THE PATENT COOPERATION TREATY (PCT)

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| <p>(51) International Patent Classification 4 : C12P 21/00, C12N 5/00 A61K 39/395, C12N 15/00</p> | <p>A1</p> | <p>(11) International Publication Number: WO 89/01975 (43) International Publication Date: 9 March 1989 (09.03.89)</p> |
| <p>(21) International Application Number: PCT/GB88/00695 (22) International Filing Date: 24 August 1988 (24.08.88) (31) Priority Application Number: 8719963 (32) Priority Date: 24 August 1987 (24.08.87) (33) Priority Country: GB (71) Applicant (for all designated States except US): CELL-TECH LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): CATTANEO, Antonino [IT/IT]; Via Antonio Musa, 12-A, I-00161 Rome (IT). NEUBERGER, Michael, Samuel [GB/GB]; 34 Bateman Street, Cambridge CB2 1NA (GB). (74) Agent: MERCER, Christopher, Paul; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).</p> | | <p>(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p> |

(54) Title: RECOMBINANT ANTIBODIES AND METHODS FOR THEIR PRODUCTION

pSV-HSV_λ1



(57) Abstract

There is provided a process for the production of an assembled polymeric recombinant antibody having its constant regions derived from an IgM, characterised in that the process is carried out in the absence of J-chain or any active gene coding therefor. Preferably, the host cell is an actively secreting cell, such as a glial cell, so that the transfor... transplanted into the brain.

Recombinant antibodies and methods for their production.

The present invention relates to the production of IgM using recombinant DNA technology.

Immunoglobulins (Igs), part of the body's normal defence system, have been well known for a considerable time. There are five classes of Ig, being IgA, IgD, IgE, IgG and IgM. When mounting an immune response to a soluble antigen, generally significant amounts of IgM are first produced. The production subsequently switches towards production of IgG. Such antibodies can be found circulating in most body fluids, such as blood and lymph fluids. However, the antibodies cannot cross the blood/brain barrier.

IgG is a monomeric species, i.e. it is found as single units comprising two heavy and two light chains bound together in conventional fashion by disulphide bonds. Since IgG is monomeric, it readily attaches to its antigen. However, it is generally unable to activate any of the body's effector functions until it is aggregated.

IgG is used in diagnosis, for instance for blood typing, but has disadvantages in that it only gives a positive result if it can be caused to aggregate. It is often difficult to ensure that the diagnostic test system is set up to produce aggregation only when a positive result should be obtained.

IgM is a pentameric species comprising five single units, similar to IgG units, bound together at the free ends of the heavy chains. In natural IgM there is a small protein molecule, known as J chain, associated with the pentameric unit at the free ends of heavy chains. There is only one J chain per pentameric unit and the J chain appears to be located between only two of the IgM single units. Conventional wisdom among immunologists is that J chain is essential for enabling expression, assembly and secretion of pentameric IgM.

Since IgM is a pentameric unit, it is effectively already aggregated. It therefore finds use in diagnostic testing and can give a more accurate result than an IgG based test. However, IgM is relatively less abundant than IgG. There is therefore a need for a more abundant supply of IgM.

In recent years, the advent of the monoclonal antibody technique and the techniques of recombinant DNA technology has enabled the production of large quantities of pure Ig. Moreover, it has enabled the production of "chimeric" antibodies, i.e. antibodies having a variable region derived from one species and a constant region derived from another species, "altered" antibodies, i.e. antibodies having a variable region from an Ig attached by peptide linkage to a co-expressed effector protein, and "humanised" antibodies, i.e. antibodies in which the complementarity determining regions (CDRs) of an antibody from one species have been grafted onto the framework regions of the variable region of an antibody from a different species, usually a human. All such antibodies are referred to herein generically as "recombinant" antibodies.

The production of such recombinant antibodies is shown, for instance, in EP-A-120 694 (Celltech), EP-A-0 125 063 (Genentech), EP-A-0 174 494 (Stanford), EP-A-0 194 276 (Celltech) and EP-A- 0 239 400 (Winter).

Although it has been suggested that such recombinant antibodies could be expressed in and secreted by any commonly available prokaryotic or eukaryotic host cell, such as microbial, yeast or mammalian host cells, it has generally been found to be necessary to use lymphoid cells to obtain efficient expression, secretion and assembly of recombinant antibodies. The most usually used host cells are myeloma or myeloma-derived host cells. It has generally been found that recombinant antibodies are not efficiently expressed, are not properly assembled or are not secreted when other commonly available host cells, such as E. coli, S. cerevisiae, CHO or HeLa cells, are used.

According to a first aspect of the present invention, there is provided a process for the production of an assembled polymeric recombinant antibody having its constant regions derived from an IgM, characterised in that the process is carried out in the absence of J-chain or any active gene coding therefor.

The present inventors have surprisingly discovered that recombinant polymeric IgM-type antibody can be produced in the absence of J-chain and that such recombinant polymeric IgM-type antibody can be expressed, assembled and secreted. This is directly contrary to the conventional wisdom. It is to be noted that in prior art procedures for producing recombinant antibodies, lymphoid cell lines were used as host cells. Such cells have endogeneous active genes encoding J-chain and

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therefore, although no specific measures may have been taken to produce J-chain, the endogeneous genes produced sufficient J-chain to enable an IgM-type molecule to be assembled in its presence.

The discovery of the present inventors means that it will be possible to express, secrete and assemble a recombinant polymeric IgM-type antibody from any convenient non-lymphoid host cell or lymphoid host cell in which the endogeneous J chain-encoding genes are absent or inactive with the use of only one or at most two expression vectors. There will be no need to produce a vector encoding the J chain. This will clearly simplify the procedure needed to produce a recombinant IgM-type antibody.

Even though it may be possible to use any available host cell for recombinant IgM-type antibody production, it is likely that, in many cases, the amount of production will be low, thus making it difficult to produce the recombinant IgM-type antibody on a commercial scale without the need to resort to the use of lymphoid host cells.

However, the present inventors have discovered that it is possible to use a glioma cell line as a host cell for the production of recombinant polymeric IgM. The glioma cell line is a cancerous cell line derived from glial cells from within the brain. Glial cells are classified as actively secreting cells. It is also possible to use other non-lymphoid actively secreting cells as host cells for the production of recombinant polymeric IgM.

Among the actively secreting cells which can be used as host cells are exocrine cells, such as pituitary or pancreatic cells, and neural cells, such as pheochromal cells and, in particular, glial cells. Such actively secreting cells have the advantage that normal (non-cancerous) cells can readily be cultured in vitro. They thus provide a host system for the production of recombinant polymeric IgM which will avoid any problems inherent in the use of cancerous host cells.

Preferably, the non-lymphoid actively secreting cells are neural cells, such as glial cells or pheochromal cells.

The advantage of using such a neural cell is that neural cells have very low amounts of or no histocompatibility antigens. They can therefore be transplanted between donors and recipients without producing any significant histocompatibility problems. Thus, a transformed neural cell could be cultured in vitro and then transplanted into a recipient so as to enable the recipient to produce in vivo the recombinant polymeric IgM. This could have significant medical application.

For instance, a neural cell transformed to enable it to produce an anti-acetylcholinesterase IgM-type antibody could be transplanted into the brain of a person suffering from Alzheimer's disease. The antibody secreted by the transformed cell may then be able to alleviate or abolish the symptoms of the disease.

Thus, a transformed neural cell could be used to circumvent the blood/brain barrier and allow the secretion in the brain of a useful IgM-type antibody, for instance directed against a brain tumour antigen or a viral antigen.

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A particular embodiment of the present invention relates to the use of a glial cell as a host cell for the production of an assembled polymeric IgM-type recombinant antibody having a specificity for acetylcholine esterase. The transformed cell line would be of use for transplantation into the brain of a person suffering from Alzheimer's disease.

The recombinant IgM-type molecule produced by the process of the present invention may be identical to a natural IgM antibody except that it does not include a J-chain. Alternatively, it may be a chimeric antibody having an IgM-type constant region. In a further alternative it may be a "humanised" antibody having mouse complementary determining regions grafted onto a human (or other non-mouse species) IgM molecule framework regions.

The present invention includes all novel plasmids, expression vectors, cell lines and products used or produced in putting the invention into effect.

Processes for producing vectors containing genes encoding all or part of an Ig chain by recombinant DNA techniques are now well known in the art. These techniques are shown, for instance, in the European patent applications referred to above. General procedures for producing and manipulating vectors and transforming cells with the vectors are also well known and described in a number of textbooks and laboratory manuals. Also well known in the art are processes for culturing transformed host cells and recovering the desired product from the culture. Such processes can readily be applied to the present invention, given the teaching of the present application.

The present invention is now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows the structure of plasmid pSV-HSV λ 1 wherein the thin line denotes the pSV2neo vector, sequences of the immunoglobulin λ 1 light chain gene are stippled, the Drosophila hsp70 promoter region (phsp70) is hatched, the leader exon derived from the heavy chain variable region (LHP) is depicted by a filled box and the open box denotes a 60 nucleotide stretch of the 5' -untranslated region of tk:

Figure 2 shows the structure of plasmid pSV-HSV μ 2 wherein the various DNA segments are depicted as for pSV-HSV λ 1 except that the vector (thin line) is pSV2gpt. The DNA encoding the constant region is derived from a cDNA encoding the C μ 1, 2, 3 and 4 domains as well as the secreted tailpiece (tp). The HS-C μ 2 transcription unit uses the SV40 polyadenylation site (SV POLY A) of pSV2gpt;

Figure 3 shows the inducible transcription of the λ gene in four pools of HeLa cells and in J558L cells;

Figure 4 shows immunoglobulin secretion from transfected cells before (-) or after (+) heat-shock and in the absence or presence of tunicamycin (+ Tm);

Figure 5 shows that IgM secreted from C5 glioma cells is polymeric;

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Figure 6 shows electron micrographs of negatively stained IgM molecules, purified on hapten sorbents, from the supernatant of heat-induced C6 [HSV μ 2/HSV μ 1] transfectants; and

Figure 7 shows an analysis for the presence of J-chain polypeptide.

In the following description various published papers are referred to by a number in brackets. A list of these papers in numerical order is given at the end of the description.

In Figures 1 and 2, the transcription start sites are indicated by an arrow and the probes used for ribonuclease protection assays (SP6-V λ and SP6-HSV μ 2) are also indicated; the SP6-HSV μ 2 probe lacks the L μ P-V μ 2 intron. Restriction sites are abbreviated: B, BamHI; Bg, BglII; H, HindIII; Ha, HaeII; Hh, HaeI; Hh, HhaI; P, PstI; Sa, Sau3AI; Sc, SacI; Sp, SphI; St, StuI; X, XhoI. Note that not all the sites for any one enzyme are shown - only those relevant to plasmid or probe construction are included.

In Figure 3, (A) shows an assay of λ 1 mRNA in HeLa transfectants. Ribonuclease protection assays were carried out using the SP6-V λ probe and cytoplasmic RNA from four pools of HeLa cells stably transfected with pSV-HSV λ 1. RNA was prepared from cells before (-) and after (+) heat shock. RNA from the plasmacytoma J558L served as a control and an end labelled HpaII digest of pBR322 provided size markers. The protected fragment (HS λ) is the length of the entire V λ exon, and (B) shows an assay of HS-V μ 2 RNA in J558L transfectant J[(HSV μ 2)]. The ribonuclease protection assay was

carried out on induced (+) and uninduced (-) cells using a probe for the 5'-end of the HS-V μ 2 transcript as well as for gpt mRNA.

Plasmids were constructed in which transcription of the regions encoding Ig heavy and light chains was under the control of the promoter of the *Drosophila* hsp70 heat-shock gene.

The Heavy Chain Plasmid

The plasmid for heavy chain expression, pSV-HSV μ 2 is shown in Figure 1 and includes most of the coding sequence of the cDNA for an IgM heavy chain with the hsp70 promoter at the 5' end and the SV40 polyadenylation signal at the 3' end. The transcription unit also contains a single intron between the regions encoding most of the leader peptide and the V_HDJ_H region. The transcription unit is linked to a gene encoding guanine phosphoribosyl transferase (gpt) which can be used as a selective marker.

Plasmid pSV-HSV μ 2 includes the HS-V μ 2 transcription unit cloned in a derivative of pSV2GPT (1) in which the BamHI site had been converted to a SacI site by use of linkers. The HS-V μ 2 transcription unit was assembled in three parts. The promoter/transcription start region was obtained from plasmid pF1 (2) as an EcoRI-BglII fragment which contains the *Drosophila* hsp70 promoter. The EcoRI-BglII fragment comprised an EcoRI-SalI fragment extending from the 5' end to immediately 3' of the TATA in the *Drosophila* hsp70 promoter. The EcoRI-SalI fragment is fused to a 60 nucleotide HaeIII-BglII fragment of the herpes simplex virus (HSV) tk gene which includes the RNA cap site.

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The DNA encoding the leader region of the heavy chain polypeptide (L_{HP}) was obtained from the mouse V_H gene V-47, as a Sau3AI-PstI fragment extending from the transcription start through the leader-V_{HP} intron to the +5 codon of the gene (3). The rest of the heavy chain coding region was derived as a PstI-HaeII fragment of the cDNA clone for the heavy chain of a NP-specific antibody pAB11 (4). The fragment extends from the +5 codon to within the C μ 3'-untranslated region.

After final assembly of pSV-HSV μ 2, the HaeII site at the 3' end of C μ is brought adjacent the SacI site in the derivatised pSV2gpt vector. Plasmid pSV-HSV μ 2 is a cDNA derivative of pSV-HSV μ 1 (5).

A probe for mapping HS-V 2 transcripts was obtained by subcloning into pSP65 a fragment derived from an intronless derivative of pSV-HSV 2 extending from the StuI site in V_{HP} to the XhoI site in the hsp70 promoter.

The Light Chain Plasmid

The plasmid for light chain expression, pSV-HSV λ 1 is shown in Figure 2. It includes the expressed, genomic λ 1 gene of the mouse myeloma HOPC 2020 but with the promoter/leader exon replaced by the hsp70 promoter/V_H-leader portion of plasmid pSV-HSV μ 2. The HSV λ 1 light chain transcription unit is linked to a gene endowing resistance to neomycin (neo) which can be used as a selective marker.

Plasmid pSV-HSV λ 1 is based on a derivative of pSV2neo (6) in which the HindIII site has been destroyed by filling in and a new HindIII site created by linker insertion in the BamHI site. The promoter/transcription

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start/leader region is the same as in pSV-HSV μ 2 except in that (i) the region 5' of the promoter extends only as far as the SphI site and (ii) the L_{MP}-V_{MP} intron is truncated at an HhaI site. This HhaI site in the L_{MP} intron is fused to a PstI site in the L λ -V λ intron of the expressed λ 1 gene of mouse plasmacytoma HOPC 2020 (7) which provides the rest of the transcription unit.

A probe for light chain expression was obtained by subcloning the BamHI-PstI V λ fragment of the HOPC 2020 light chain gene into plasmid pSP65.

Cell Lines and Transfection

Mouse plasmacytoma cell line J558L (8), its IgM secreting transfectant JW1/2/2 (9), rat myeloma cell line YO (10), rat glioma cell line C6 (11), CHO cells and HeLa cells were grown in DMEM containing 10% FCS. Pheochromocytoma cells PC12 (12) were grown in RPMI medium containing 10% heat-inactivated horse serum and 5% FCS.

The phenotype of the C6 glioma cells was confirmed by: (i) measuring receptors for nerve growth factor (NGF) (13); fluorescent staining with antibody MC 192 (14); immunoprecipitation of S100 protein (15); and immunofluorescent staining for glial fibrillar acidic protein (16). The PC12 cells were shown to respond to NGF.

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Transfection of J558L and C6 glioma cells was achieved using spheroplast fusion, carried out as described by Neuberger (3). Transfection of PC12 cells was achieved using electroporation performed by giving four 2kV pulses to a suspension of 2.5×10^7 cells in 0.5 ml of supercoiled plasmid DNA (17). Transfection of HeLa and CHO cells was achieved by calcium-phosphate coprecipitation.

For selection of neo transfectants, G418 was used at 0.5 mg/ml (PC12), 1 mg/ml (C6) or 2 mg/ml (other cells). gpt colonies were selected as described by Mulligan and Berg (1) for non-lymphoid cells and as described by Neuberger et al. (9) for J558L cells.

Analysis of RNA

Total cytoplasmic RNA was prepared as previously described by Neuberger (3). For heat induction, RNA was extracted 4 hours after the cells had been subjected to 2 hour shock at 42.5°C. Ribonuclease protection assays were performed as described by Melton et al. (18).

Analysis of Protein

For biosynthetic labelling of immunoglobulin, cells (5×10^6 in 1 ml) were heat-shocked at 42.5°C for 2 hours, washed and resuspended in medium containing 5% dialysed FCS, one-tenth the normal concentration of methionine and 50 μ Ci/ml L- 35 S]methionine (1200 ci/mmol; Amersham International). After a 14 - 16 h incubation at 37°C, cells were collected by centrifugation, washed and lysed in 0.2 ml PBS/0.5% Nonidet-P40/1mM phenylmethylsulphonyl fluoride (PMSF).

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For analysis of J chain, the labelling period was shorter and cells were lysed in 0.1M-Tris.HCl (pH 8.0) / 0.1M-KCl / 5 mM-MgCl₂ 0.5% NP40 / 1mM-PMSF. When required, tunicamycin (8 μ g/ml) was included during the heat-shock and labelling periods.

Immunoprecipitations from culture supernatants or cytoplasmic extracts were carried out by incubation and purified rabbit anti-mouse μ antiserum or rat monoclonal antibody SM1/45, goat anti-mouse λ monoclonal anti-idiotypic antibodies Ac38 and Ac146 (19) or rabbit anti-mouse J chain antiserum, followed by precipitation with protein A-Sepharose, (Pharmacia). After washing with 50 mM-Tris.HCl (pH 7.6) / 1M-NaCl / 0.25% NP40, samples were dissolved in reducing SDS sample buffer and analysed by electrophoresis through SDS/polyacrylamide gels (23). Unreduced samples were analysed on 4% acrylamide / 0.7% N,N'-diallyltartardiamide gels (24). Purification on hapten sorbents was carried out using NIP-caproate linked to Sepharose as previously described (9).

For analysis in the electron microscope, antibody that had been freshly affinity purified on hapten columns was concentrated to 1 mg/ml, layered onto carbon films and viewed by negative contrast following staining with uranyl acetate.

Cell lines were transfected with either plasmid pSV-HSV μ 2 alone (J558L) or with plasmids pSV-HSV μ 2 and pSV-HSV λ 1 together (other cells). Association of the polypeptides encoded by the two plasmids should yield an IgM λ 1 antibody with specificity for the hapten 4-hydroxy-3-nitrophenacetyl (NP). Stable transfectants

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were screened for the expression of Ig gene mRNA and protein. The results are shown in Table 1, where they are compared with the result from the JW1/2/2 line which secretes IgM constitutively.

Table 1

Antibody yields from transfected cells.

| Cell Line | Cell-type | Concentration of IGM μ g.ml). |
|-----------|------------------|--------------------------------------|
| J558L3 | plasmacytoma | 0.5 |
| C6 | glioma | 0.6 |
| PC12 | Pheochromocytoma | 0.01 |
| CHO | Fibroblast | 0.01 |
| HeLa | Epithelial | 0.005 |
| JW1/2/2 | Plasmacytoma | 3.5 |

3×10^6 cells/ml (initial concentration) were incubated at 37°C for 3 days with 1 hour heat shocks at 42.5°C every 24 hours. The concentration of IgM was determined by radioimmunoassay on antigen-coated plates using purified JW1/2/2 IgM for calibration.

Four different pools of HeLa cells were transfected with pSV-HSV λ 1 and three showed inducible λ gene

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transcription. The fourth pool transcribed the gene constitutively. These results are shown in Figure 3A. Ribonuclease protection assays (18) were carried out using the SP6-V λ probe and cytoplasmic RNA from the pools of HeLa cells stably transfected with pSV-HSV λ 1. RNA was prepared from cells before (-) and after (+) heat shock. RNA from J558L served as a control and an end-labelled HpaII digest of pBR322 provided size markers. The protected fragment (HS λ) is the length of the entire V λ exon.

Similarly, pSV-HSV μ 2 transfectants of J558L cells showed inducible transcription of the inducible heavy chain gene. The ribonuclease protection assay was carried out on induced (+) and non-induced (-) cells using the probe for the 5' end of the HS-V μ 2 as well as a probe for gpt mRNA. The size of the fragment obtained in the RNase protection assay indicates that the transcripts were initiated by the Hsp70 promoter. The result is shown in Figure 3B.

It was confirmed by Northern blotting that the HSV μ 2 and HSV λ 1 transcripts were of the expected size.

Secretion of glycosylated IgM by non-lymphoid transfectants

Analysis of cells transfected with plasmid pSV-HSV λ 1 revealed that most of these transfectants secreted λ 1 light chains into the culture supernatant. Clones of the transfected rat pheochromocytoma cell line PC12 and of the rat glioma cell line C6 were analysed for polypeptide synthesis. Whilst there was clonal

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variation in the amount of λ expressed (some neo⁺ clones being negative for λ 1 expression), it was clear from immunoprecipitation experiments that heat induction of many of the C6 and PC12 transfectants resulted in the secretion of light chains. The results are shown in Figure 4A which shows light chain secretion by J558L and C6 and by pSV-HSV λ 1 transfectants of C6 and PC12. Samples were immunoprecipitated from culture supernatants using anti-antiserum. Interestingly, the amount secreted by these transfectants is comparable to the amount of constitutive light chain secretion by the mouse plasmacytoma J558L, despite the fact that J558L contains appreciably more λ 1 mRNA.

In order to test whether the pheochromocytoma and glioma lines were able to assemble complete immunoglobulin, biosynthetically labelled proteins of PC12 and C6 cells that had been transfected with both pSV-HSV μ 2 and pSV-HSV λ 1 were analysed. The results are shown in Figure 4B which shows intracellular immunoglobulin production in PC12 and C6 expression plasmids. After biosynthetic labelling, cytoplasmic samples were immunoprecipitated with anti- μ antiserum. These results show that heat shocking of both cell lines results in induction of the synthesis of μ polypeptide. The μ heavy chain is associated with λ light chain and is glycosylated as judged by the effect of the glycosylation inhibitor tunicamycin.

The C6 glioma transfectants were able to secrete the associated μ and λ polypeptides. This is shown by Figure 4C which shows the secretion of IgM by C6[HSV 1/HSV μ 2] transfectant and by constitutive

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IgM-secreting J558L transfectant, JW1/2/2. After biosynthetic labelling, samples from the supernatant were precipitated with anti- μ antiserum. The high molecular weight band in this gel that is indicated by an arrow is also precipitated from untransfected C6 cells by anti- μ antibodies and reflects serological cross-reaction. The other arrow indicates the dye-front. The antibody secreted by these glioma transfectants was similar to that secreted by the plasmacytoma transfectant JW1/2/2 in that both antibodies bound the hapten NP. This is shown in Figure 4D which is a comparison of IgM secretion by JW1/2/2 (constitutive plasmacytoma expression), C6[HSV λ 1/HSV μ 2] (heat-inducible glioma transfectant) and J[HSV μ 2] (which is a pSV-HSV μ 2 transfectant of J558L and is therefore a heat-inducible IgM secreter). All samples were prepared following heat shock and analysed in parallel except that only half the JW1/2/2 sample was loaded on the gel and an incubation of J[HSV μ 2] cells in the absence of heat-shock was performed for the right hand lane. IgM from culture supernatants was purified by adsorption onto NIPcap-Sepharose and eluted with 1 mM-NIPcapOH.

Both antibodies were recognized by anti-idiotypic antibodies. Figure 4E shows immunoprecipitation of glioma IgM with anti-idiotypic antibodies. Immunoglobulin in the supernatant of biosynthetically labelled C6[HSV μ 2/HSV μ 1] cells prior to (-) or following (+) heat shock was precipitated using monoclonal anti-idiotypic antibody Ac38 or Ac146 (19). Furthermore, the extent of glycosylation of the μ polypeptide secreted by the two cell-types appears similar as judged by mobility in SDS/polyacrylamide gels.

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To compare the efficiency of antibody secretion by a glioma transfectant to that by a plasmacytoma, we used the C6 double transfectant C6[HSV μ 2/HSV λ 1] and the J558L transfectant J[HSV μ 2], which expresses an inducible μ gene from the transfected pSV-HSV 2 plasmid and constitutively transcribes its endogenous λ gene. In both transfectants, heat shocking leads to induction of μ synthesis in at least 80% of the cells as judged by cytoplasmic immunofluorescence. Following periodic heat pulses, the concentration of hapten-specific IgM in the culture supernatants was estimated by radioimmunoassay.

The results (Table 1) confirm that the level of inducible secretion is similar in the glioma and plasmacytoma transfectants - a conclusion which is in keeping with the results of biosynthetic labelling (Figure 4D). However, it is worth noting that the antibody yields obtained from the HeLa and CHO cell transfectants were considerably lower (Table 1).

IgM secreted by glioma transfectants is polymeric and does not contain J chain.

As the conventional wisdom is that J chain is necessary for IgM assembly and secretion and as J chain expression appears to be restricted to mature cells of the B lymphocyte lineage, it was important to ascertain whether the IgM secreted by the glioma transfectants contained immunoglobulin J chain.

To determine whether there was any intracellular J chain in C6 cells, immunoprecipitation was carried with an anti-J chain antiserum using cytoplasmic extracts of

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00695

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| I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁴ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁴ : C 12 P 21/00; C 12 N 5/00; A 61 K 39/395; C 12 N 15/00 | | |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| IPC ⁴ | C 12 N; C 12 P | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ | | |
| Category ⁹ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | Nature, volume 311, -6 September 1984, P. Brandtzaeg et al.: "Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immuno- globulins", pages 71-73 see page 71, column 2, paragraph 3; table 1 last line | 1,9,10 |
| A | Gene, volume 51, no. 1, April 1987, Elsevier Science Publishers B.V. (Biomedical Division), U.H. Weidle et al.: "Reconstitution of functionally active antibody directed against creatine kinase from separately expressed heavy and light chains in non-lymphoid cells", pages 21-29 see the whole article | 1-4,8-10 |
| A | Nature, volume 314, 4 April 1985, | 1-3,9,10 |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance.</p> <p>"E" earlier document but published on or after the international filing date.</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means.</p> <p>"P" document published prior to the international filing date but later than the priority date claimed.</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention.</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step.</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family.</p> </div> </div> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | | Date of Mailing of this International Search Report |
| 2nd December 1988 | | 29 DEC 1988 |
| International Searching Authority | | Signature of Authorized Officer |
| EUROPEAN PATENT OFFICE | | [Signature] |

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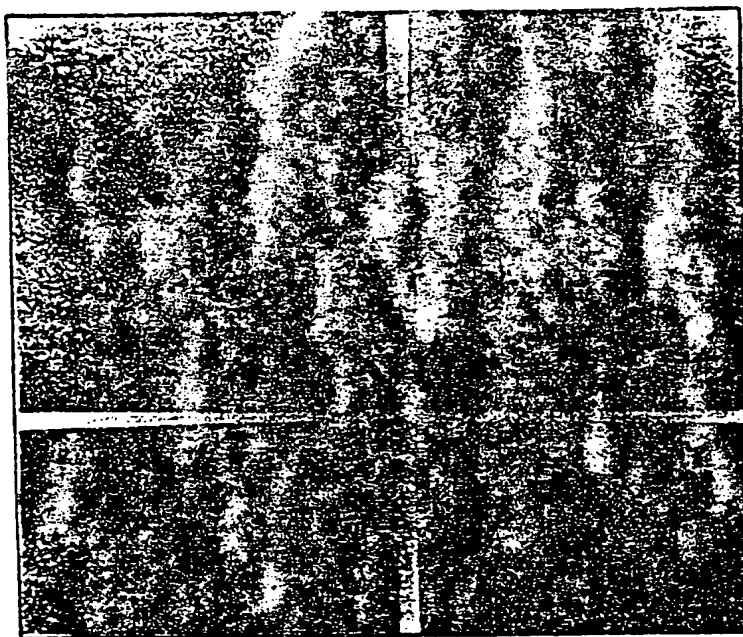
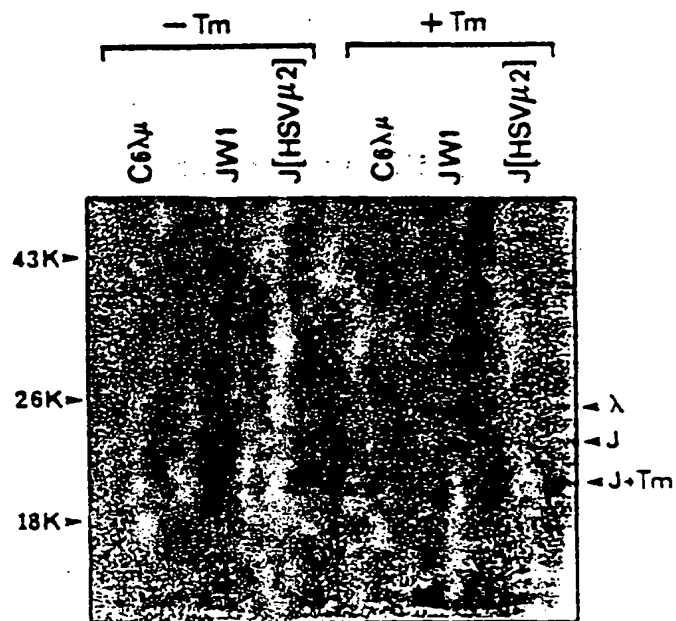
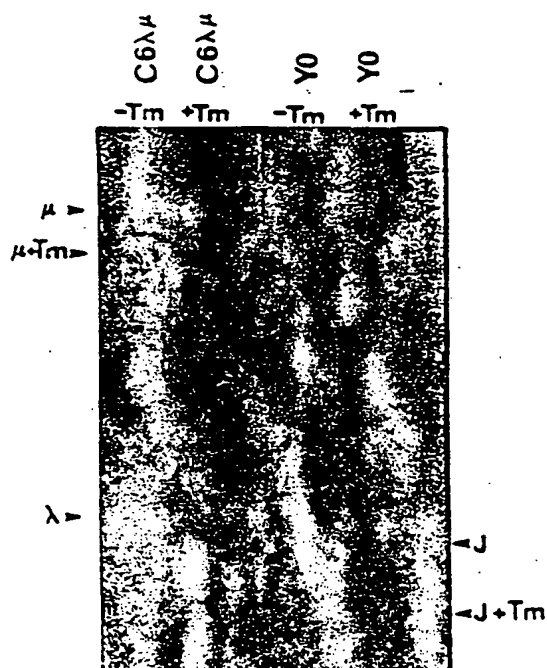


FIG. 6

A

FIG. 7

B



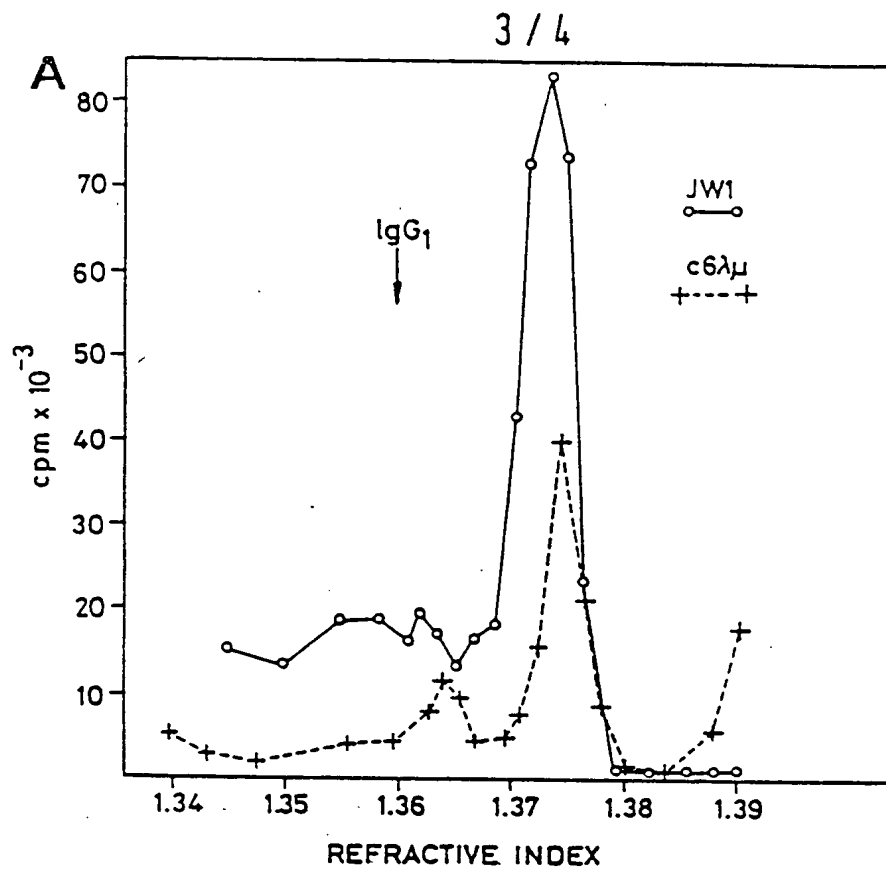
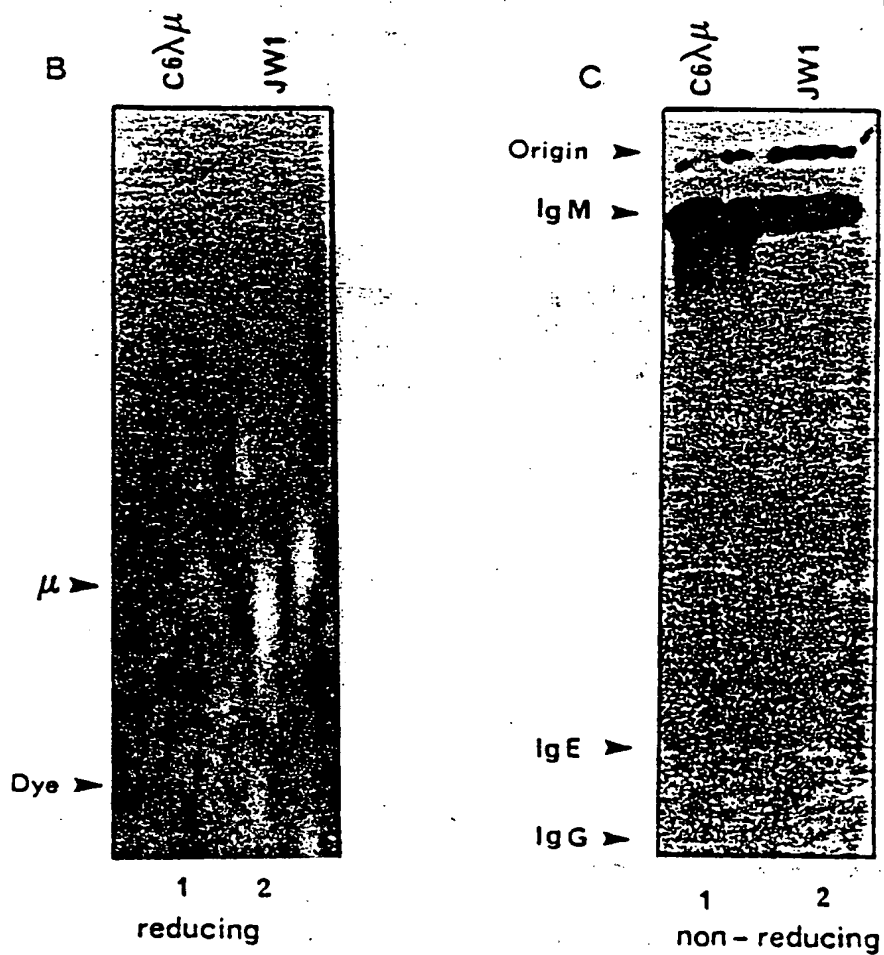
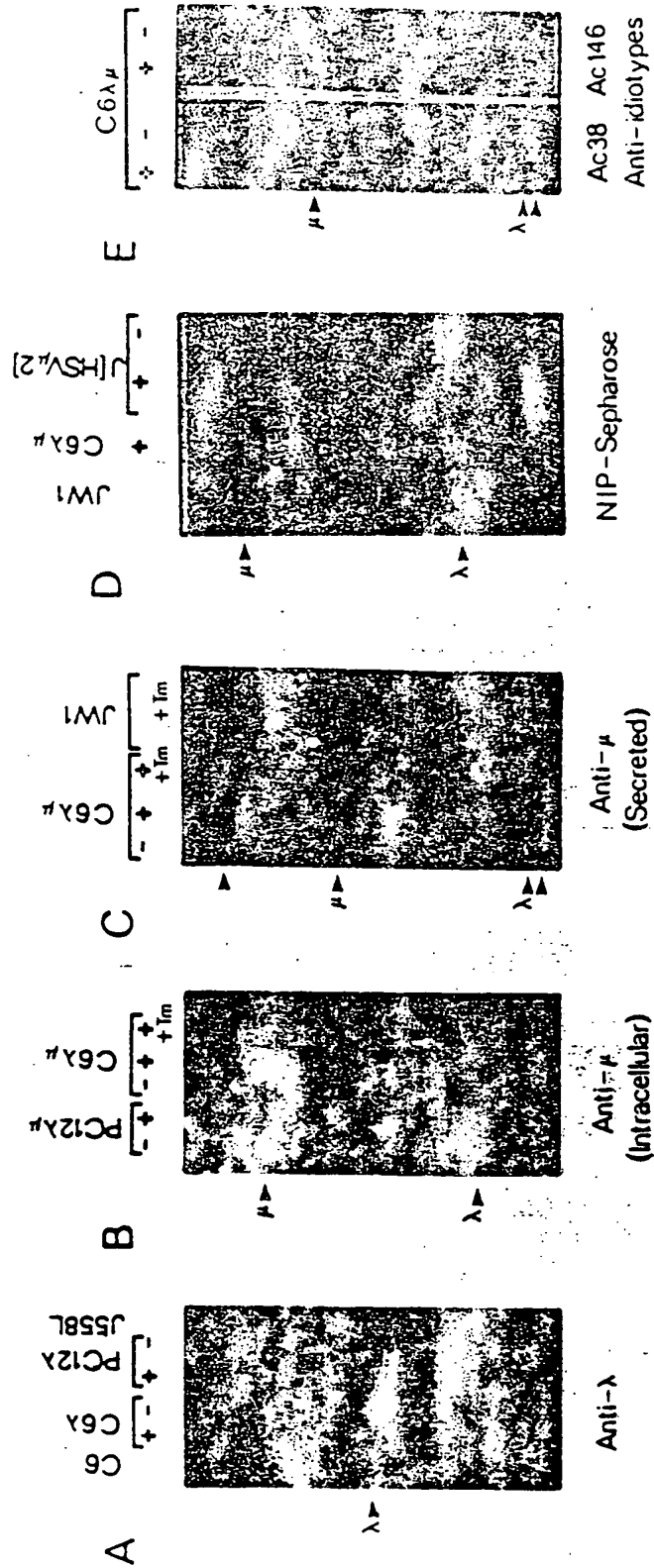


FIG. 5



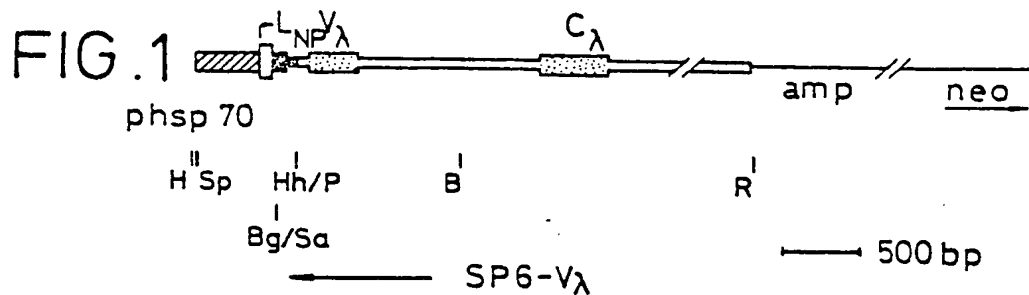
2 / 4

FIG. 4



pSV-HSV λ 1

1 / 4



pSV-HSV μ 2

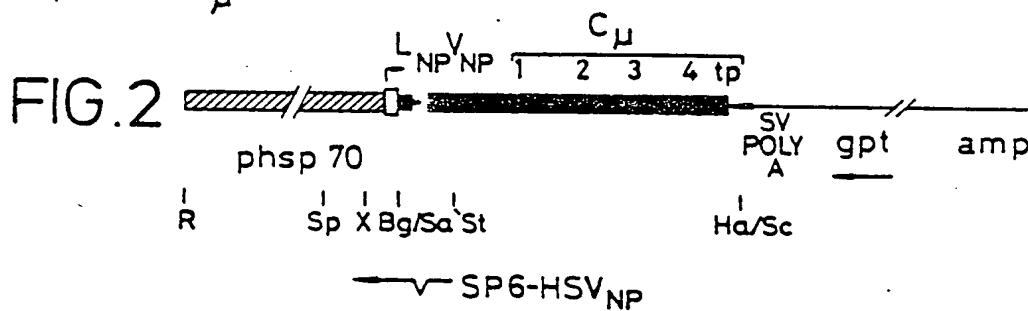
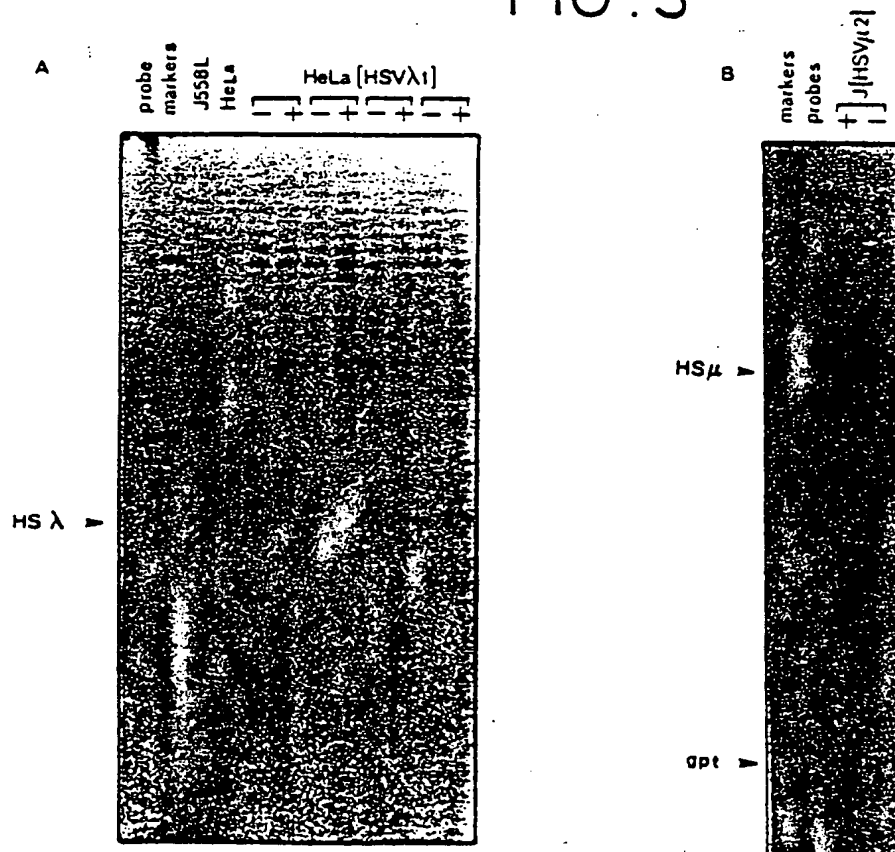


FIG. 3



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8. A non-lymphoid actively secreting cell which has been transformed such that it can express an IgM-type antibody.

9. An IgM-type antibody substantially free of J-chain.

10. The IgM-type antibody of claim 9 which is a natural, chimeric or humanised antibody.

CLAIMS

1. A process for the production of an assembled polymeric recombinant antibody having its constant regions derived from an IgM, (or IgM-type antibody), characterised in that the process is carried out in the absence of J-chain or any active gene coding therefor.
 2. The process of claim 1, wherein a host cell lacking a J-chain encoding gene or having an inactive J-chain encoding gene is transformed with a vector containing a gene encoding an IgM-type heavy chain and a gene encoding a complementary light chain.
 3. The process of claim 1, wherein a host cell lacking a J-chain encoding gene or having an inactive J-chain encoding gene is transformed with a vector containing a gene encoding an IgM-type heavy chain and a vector encoding a complementary light chain.
 4. The process of any one of claims 1 to 3, wherein the host cell is a non-lymphoid actively secreting cell.
 5. The process of claim 4, wherein the actively secreting cell is an exocrine cell or a neural cell.
 6. The process of claim 5, wherein the actively secreting cell is a glial cell.
 7. The process of any one of claims 1 to 6, wherein the IgM-type antibody has an anti-acetylcholine esterase specificity.
- over*

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Benda

that J chain is required for the assembly and secretion of IgM. IgM secreted by the glioma transfectants is part pentameric, part hexameric and it is conceivable that the presence of hexamers is a direct consequence of the absence of J chain. Therefore it is clear that J chain is not required for the production of polymeric IgM in good yield from the glioma transfectants.

Both PC12 and C6 cells are derived from cell types present in the nervous system. The fact that transfectants of both cell types are able to synthesize and secrete immunoglobulin means that it should be possible to engineer the production of specific antibodies in the central nervous system of transgenic organisms in order to perturb or modulate the activity of selected neuronal pathways or to effect medication.

It will of course be appreciated that the present invention has been described above purely by way of illustration and that modifications and alterations of detail can be made by the skilled man without departing from the scope of the invention.

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Finally, it worth noting that pulse-chase experiments performed using biosynthetic labelling revealed that the polymerization of the IgM molecules occurred prior to their secretion from the glioma or plasmacytoma cell.

Discussion

Several different non-lymphoid cell-lines were transfected with immunoglobulin heavy and light chain genes whose expression was under control of a heat-shock promoter. Transfectants were obtained that showed inducible expression, demonstrating that non-lymphoid cells are capable of assembling, processing and secreting immunoglobulin. There was considerable variation in the yield of immunoglobulin obtained using different cell-types as host. Whereas HeLa and CHO cells gave relatively poor yields, the amounts of antibody secreted by glioma and pheochromocytoma transfectants were comparable to those obtained using a plasmacytoma host. The greater efficacy of C6 glioma or PC12 as opposed to CHO or HeLa transfectants in antibody secretion does not appear to be due to a difference in the induction of immunoglobulin mRNA synthesis but reflect the fact that the glioma and pheochromocytoma are actively secreting cell types and may therefore be better equipped for the production of secreted immunoglobulin.

The IgM secreted by the C6 transfectants is polymeric, notwithstanding the absence of immunoglobulin J chain. This was somewhat unexpected as it has been proposed

mvr

In both analyses, the IgM secreted by the glioma transfectant behaved exactly like the IgM secreted by the plasmacytoma and revealed itself to be a high molecular weight, covalently-associated aggregate. The resolution of the centrifugation and gel electrophoresis experiments was not, however, sufficient to demonstrate that the polymeric IgM secreted by the glioma transfectants was of exactly the same size as that produced by the plasmacytoma cells. An analysis of negatively stained protein samples was carried out using electron microscopy, the results of which are shown in Figure 6. Different fields are shown in which both hexamers (H) and pentamers (P) are visible. Comparison of the IgM samples prepared from plasmacytoma and glioma transfectants shows that whereas the IgM secreted by the plasmacytoma is almost exclusively pentameric, the sample prepared from the glioma transfectants is found to contain both cyclic hexamers and cyclic pentamers in approximately equal amounts. The sample prepared from C6 cells also contains some molecules which, at first sight, appear to be incompletely assembled structures. However, the same structures are seen in the plasmacytoma IgM sample and are most probably hexamers or pentamers that are not lying flat on the grid (22). Analysis of IgM secreted from the heat-inducible plasmacytoma transfectant J[HSV μ 2] revealed it to be exclusively pentameric, indicating that the presence of hexamers in the glioma sample is unlikely to be an artefact of heat shocking.

by primer extension assays. Furthermore, analysis of IgM secreted by the plasmacytoma and C6 transfectants in alkaline/urea polyacrylamide gels after reduction and alkylation revealed J chain to be present only in the plasmacytoma sample.

The sizes of the IgM secreted by the plasmacytoma and glioma transfectants were compared using both sucrose gradient centrifugation (Figure 5A) and electrophoresis through non-reducing SDS/polyacrylamide gels (Figure 5B)

In the Analysis by sucrose gradient centrifugation, supernatants (1 ml) from biosynthetically labelled C6[HSV μ 2/HSV λ 1] (+-----+), plasmacytoma IgM-secreting transfectant JW1/2/2 (o—o) or IgG1-secreting hybridoma P8.86.9 were loaded onto a gradient (15ml) of 10% sucrose in PBS and subjected to centrifugation in a Beckman SW40 rotor at 4°C for 18 hours. Samples were collected, the refractive indices determined and anti-NP antibody measured by radioimmunoassay. The supernatants from the C6 and J558L transfectants were analysed in parallel.

In the analysis by polyacrylamide gel electrophoresis, anti-NP antibody in the supernatants of biosynthetically labelled JW1/2/2 or of heat-induced C6[HSV μ 2/HSV λ 1] was purified on hapten sorbents and analysed either after reduction (left panel) on a 7.5% SDS/polyacrylamide gel or unreduced (right panel) on a 4% polyacrylamide gel made using N,N'-diallyltartardiamide to cross-link. The positions of the origin, IgM, IgE, IgG, and μ markers are indicated.

msh

biosynthetically labelled cells. The results are shown in Figures 7A and 7B. A rabbit antiserum against J chain was used to precipitate J chain from biosynthetically labelled cell extracts that had been made in the presence or absence of tunicamycin (Tm) and that had been pre-cleared with anti-light and anti-heavy chain antisera and protein A-Sepharose. Nevertheless, it will be seen that precipitation with the anti-J chain antiserum does bring down some residual immunoglobulin polypeptides. Figure 7A shows the results obtained with Rat YO myeloma cells and cells of the C6 [HSV μ 2/HSV 1] transfectant that had been heat-induced 5 hours previously were incubated in labelling medium for 5 hours. Figure 7B shows the results obtained with JW1/2/2 cells and cells of the J[HSV μ 2] and C6[HSV μ 2/HSV λ 1] transfectants that had been heat-induced 7 hours previously and were incubated in labelling medium for 1 hour.

These results show that J chain can be detected in extracts of the plasmacytoma transfectants but not in the C6 glioma sample. Various different labelling times were used as it has been reported (21) that the half-life of J chain differs considerably in different cell-lines. The detection of J chain polypeptide in the YO rat myeloma demonstrates that the anti-mouse J chain antiserum that was used will indeed precipitate rat J chain antiserum that was used will indeed precipitate rat J chain - a point that needed confirming as C6 is a glioma of rat origin. Not only was no J chain polypeptide detected in C6 cells, but it also appeared that these cells did not contain J chain mRNA as judged

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|------------|---|----------------------|
| P,X | <p>C.R. Wood et al.: "The synthesis and <u>in vivo</u> assembly of functional antibodies in yeast", pages 446-449 see page 448, paragraph 1</p> <p>The EMBO Journal, volume 6, no. 9, September 1987, IRL Press Ltd, (Oxford, GB),</p> <p>A. Cattaneo et al.: "Polymeric immunoglobulin M is secreted by transfectants of non-lymphoid cells in the absence of immunoglobulin J chain", pages 2753-2758 see the whole article</p> | 1-6,8-10 |

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